TREATMENT OF LASER INDUCED RETINAL INJURIES

ANNUAL REPORT

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June 7, 1987

Supported by

U.S. Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Grant No. DAMD17-85-G-5013

Tel-Aviv University
Ramat-Aviv, Tel-Aviv, Israel 69978

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release: distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Tel-Aviv University; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Ramat-Aviv, Tel-Aviv, ISRAEL 69978	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: USAMRDC; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-85-G-5013
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3MI-61102B510; TASK NO. CF; WORK UNIT ACCESSION NO. 446

11. TITLE (Include Security Classification)

TREATMENT OF LASER INDUCED RETINAL INJURIES

12. PERSONAL AUTHOR(S)

M. BELKIN N. NAVEH

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM Feb.86 TO Jan.87	1987 June 9	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
20	05	
06	16	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Prostaglandin E_2 (PGE_2), Vitreal protein levels Vitreal PGE_2 levels, Blood retinal barrier, Argon laser Neodymium:YAG laser, leukotriens B_4.

ABSTRACT (Continue on reverse if necessary and identify by block number)

This year's study consisted of two parts:

1. Completion of the study on steroid treatment of single argon laser.

2. Study on Neodymium:YAG (Nd:YAG) laser induce retinal damage and its relationship with prostaglandin E_2 (PGE_2) and leukotrienes response.

1. The steroid treatment study investigated the effect of steroid treatment of eyes with a single argon laser induced retinal burn on

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassied
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mrs. Judy Pawlus	22b. TELEPHONE (Include Area Code): 301/663-7325; 22c. OFFICE SYMBOL: SGRD-RMI-S

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

changes in PGE_2 production by the retina/choroid, its accumulation in the vitreous as well as vitreal protein levels indicative of blood retinal barrier (BRB) permeability changes. This study also compared the response of a single argon laser retinal burn with that of the noncoherent light exposure used during laser irradiation.

Laser induced retinal trauma was associated with an accumulation of PGE_2 and protein in the vitreous, a phenomemon not occurring in normal eyes, which is most indicative of an inflammatory reaction. Both PGE_2 and protein accumulation in the laser exposed eyes are the result of a "break" in the BRB characteristic of most inflammatory reactions. The elevation in vitreal PGE_2 levels was related with an increase in PGE_2 production by the retina/choroid. Steroid treatment effectively inhibited the laser induced inflammatory reaction as in the treated group. Normal vitreal PGE_2 and protein levels were maintained throughout a two week period following exposure. The inhibitory effect of steroid treatment on the inflammatory reaction in laser exposed eyes at the initial phase after exposure was related to reduction in PGE_2 production by the retina/choroid, thus indicating the efficacy of steroid treatment when started immidiately after exposure. However, steroid anti-inflammatory effect on the later stages following laser exposure are as yet unclear.

2.Neodymium:YAG (Nd:YAG) laser induced retinal injury study - In this study, the effect of a single Nd:YAG laser induced retinal burn on PGE_2 and leukotriene B_4 response was investigated. Nd:YAG laser retinal exposure was associated with an inflammmatory reaction of

higher magnitude and longer duration than that of the argon exposed eyes, as manifested by elevation of the levels of PGE_2, leukotrienes, and protein in the vitreous. The elevation in vitreal PGE_2 and protein levels in Nd:YAG laser exposed eyes persisted during a two week period following exposure. Likewise, the accumulation of leukotreine B_4 in the vitreous of exposed eye persisted during a two week period and was more pronounced than that of the argon group where levels peaked only once. In view of this observation, a more effective anti-inflammatory therapy of Nd:YAG laser exposed eyes directed at inhibiting leukotriene production should be considered. The Nd:YAG laser induced retinal damage is liable to be the most expected military laser induced eye injury, and our finding of a pronounced leukotriene response in Nd:YAG exposed eyes indicates the possible beneficiary effect of antileukotriene drugs in their treatment.

The clinical significance of our study lies in the demonstration of the efficacy of steroid treatment in suppression of the laser induced inflammatory reaction when started immediately after exposure. It also indicated the need for a search for other nonsteroidal anti-nflammatory medications to completely inhibit the late phases of the laser induced inflammatory response. Our finding that Nd:YAG induced inflammmatory reaction was of higher magnitude than that of argon exposed eyes, and involved a pronounced leukotriene response has a military importance indicating that antileukotriene drugs should be sought for the treatment of Nd:YAG laser exposed eyes.

Foreword

In conducting the research describe in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23 Revised 1985).

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Distribution List

Our work during the last year (1986) consisted of:

1. Study on steroid treatment of a single argon laser induced retinal lesion. - completed.

We find it appropriate to present herein all the results concerning steroid treatment of a single argon laser retinal burn, a study started during 1985 and completed in 1986. This summation enables us to draw relevant conclusions that might have a bearing on future studies and point to possible clinical application.

2. Study on leukotrienes involvement in a single argon laser induced retinal burn.
3. Study of the histopathological changes of argon laser induced retinal burns.
4. Preliminary study of prostanoid and leukotrienes involvement in a single neodymium YAG (Nd:YAG) laser induced retinal burn.

Introduction:

Study on steroid treatment of a single argon laser induced retinal burn - completed.

Introduction:

A laser lesion site in the eye is surrounded by a severe inflammatory reaction which partially underlies the incapacitation resulting from the laser injury. The laser induced inflammatory reaction which later undergoes scarring is closely related to an elevation in prostaglandin type E_2 (PGE_2) production by the exposed

retina/choroid as has been shown by us. (Annual Report 1985)

In our study, argon laser irradiation of the retina at various energy levels was associated with an increase in PGE_2 production by the retina/choroid and an accumulation of PGE_2 and protein in the vitreous body to above baseline levels. Noncoherent light exposure also elicited an increase in PGE_2 production by the retina/choroid and its accumulation in the vitreous which was lower than that observed in the laser group.

Inflammation of the eye and the associated incapacitation can be reduced by inhibition of prostaglandins production using steroids or aspirin - like drugs. Our study during 1985 on steroid treatment of a single argon laser induced retinal burn showed that daily intramuscular injection of steroids abolished the accumulation of PGE_2 and protein in the vitreous, indicative of the inflammatory reaction. During 1986 the study of the effect of steroid treatment on PGE_2 response following a single argon laser induced retinal burn was completed so it included more follow up data over a two week period after exposure.

Methodology

Pigmented Dutch rabbits of either sex weighing 1.5 to 2.0 kg were used. All animals were anesthetized with 35 mg/kg ketamine and 5 mg/kg xylazine.

Argon laser exposure. A continuous wave argon laser was used (Lasertek, 265 Excitor). The laser beam was focused on the retina

through a Goldmann coated lens, following pupil dilatation with Tropicamide 0.5%, and local anesthesia with Benoxinate 0.1%. Laser exposure of the right eye of each rabbit consisted of a single burn at a power setting of 200 milliwatt, a spot size of 500 and a duration of 0.5 seconds, aimed nasally to the optic disc, resulting in an ophthalmoscopically visible burn.

Noncoherent light exposure (Sham exposure) - Noncoherent light exposure involved pupil dilatation,contact lens fitting and exposure to the noncoherent light of the slit lamp attached to the argon laser, but without laser irradiation. Noncoherent light exposure lasted for a period similar to that used to produce a single laser application, and was identical to the prelaser procedure undergone by the experimental group but without irradiation.

Steroid treatment procedure. Dexamethasone, 0.5 mg/kg weight intramuscularly, was started during the first hour after laser exposure and was repeated daily, until 24 hours prior to sacrifice.

Sample preparation. Following enucleation, the cornea was cut all around the limbus, and the iris and ciliary body were removed by pulling gently at the iris base. The lens was easily discarded, and the vitreous was expelled by rotating the eye and inverting it so that the sclera faced the inside. The inverted globe was mounted on a conus tube, and the vitreous attachments to the optic nerve head were excised. The separated vitreous body from each animal was placed in a vial. The attached retina/choroid was then separated

carefully from the sclera using blunt scissors, and was placed in another vial containing buffer.

Prostaglanden E_2 determination - The retina/choroid preparation was incubated in 0.6 ml Krebs Ringer Bicarbonate Heppes buffer, pH 7.4 in a slow shaking bath at 37°C for 15 minutes. At the end of the incubation period, the tissue was removed, and samples from the incubation media were withdrawn for PGE_2 determination.

The vitreous body was incubated in 1.0 ml of the same buffer in a slow shaking bath at 37°C for 15 minutes and a sample was withdrawn from the media for PGE_2 determination. PGE_2 levels in each sample were determined using a radioimmunoassay with a specific antibody to PGE_2, as performed during the 1984 and 1985 study.

Protein determination Protein was measured in the vitreous body using the modified Lowery method. However, protein determination during this year was performed in a Tris buffer, as compared with a Krebs Heppes buffer used during 1985. With the latter buffer, protein levels were higher and corrections had to be made accordingly for the previous year's data. Protein levels given in this year's report have been either corrected, or determined according to the new procedure.

Experimental design. The study consisted of four groups of age and sex matched rabbits, raised in alternating 12 hour periods of light and dark, using regular fluorescent light source.

1) Control group-30 rabbits that were not exposed to either the laser irradiation or the noncoherent light illumination and had no ocular pathology.

2) Untreated argon laser (coherent light) exposed group- 73 rabbits with a single argon laser induced retinal burn, which were divided into 4 subgroups

a) 20 rabbits studied 1 day after exposure.

b) 20 rabbits studied 3 days after exposure.

c) 10 rabbits studied 7 days after exposure.

d) 23 rabbits studied 14 days after exposure.

3) Steroid treated argon laser exposed group-57 rabbits, with a single argon laser induced retinal burn who were treated with steroids according to a procedure described above. This group was also divided into 4 subgroups:

a) 12 rabbits studied 1 day after exposure.

b) 22 rabbits studied 3 days after exposure.

c) 11 rabbits studied 7 days after exposure.

d) 12 rabbits studied 14 days after exposure.

4) Noncoherent light exposed group (Sham exposure)-72 rabbits which underwent noncoherent light exposure, and were divided into 4 subgroups:

a) 21 rabbits studied 1 day after exposure.

b) 10 rabbits studied 3 days after exposure.

c) 10 rabbits studied 7 days after exposure.

d) 31 rabbits studied 14 days after exposure.

Results

PGE_2 production by retina-choroid

Control group (Table 1)-PGE_2 production by retina/choroid in this group was 360 ± 137 pg/mg weight and was considered as baseline level.

Untreated laser exposed eyes in this group PGE_2 production showed during a two week period, a biphasic elevation above baseline levels; A transitory initial peak on day 1 followed by a higher peak on day 7, (778 ± 232 pg/mg weight and 1971 ± 291 pg/mg weight, respectively), after which the levels progressively declined, but were still significantly higher than baseline (637 ± 267 pg/mg weight, $p<0.003$). Noncoherent light exposed eyes; In this group changes in PGE_2 production during the two week period followed a similar pattern to that observed in the untreated laser group but with lower peak levels (982 ± 327 pg/mg weight and 1971 ± 291 pg/mg weight, respectively, $p<0.000$). Steroid treated laser exposed eyes; Steroid treatment abolished the initial peak in PGE_2 production observed in the other two groups, and reduced the second production peak on day 7 to lower levels than in the corresponding untreated laser group (1260 ± 514 pg/mg weight and 1971 ± 291 pg/mg weight, respectively $p<0.000$). However, on day 14 further rise of production was observed in the

steroid treated group unlike the other two groups in which levels were already declining at this time.

Vitreal PGE_2 levels (Table 2) -

control group; In this group PGE_2 levels were 7476 ± 4770 pg/gm weight, and were considered as baseline.

Laser and noncoherent light exposed eyes: In both groups vitreal PGE_2 content during a two week period was similarly enhanced above baseline levels only once (day 7), (13668 ± 7125 pg/gm weight and 13460 ± 5129 pg/gm weight, respectively). Steroid treated group; In this group vitreal PGE_2 content did not exceed baseline levels at any of the time intervals during observation period,indicating that steroid treatment abolished vitreal PGE_2 accumulation.

Vitreal Protein levels (Table 3): In the control group vitreal protein levels were 1.03 ± 0.2 µg/mg weight and were considered as baseline. Following laser exposure vitreal protein content during a two week period was elevated twice, on day 3 and 14 and reached values of 0.83 ± 0.24 µg/mg weight and 0.99 ± 0.21 µg/mg weight respectively, which were significantly higher than baseline levels ($p<0.03$ and $p<0.004$, respectively). In the noncoherent light exposed eyes, vitreal protein levels were elevated above baseline only on day 1 after exposure (1.03 ± 0.3 µg/mg).

In steroid treated laser exposed eyes, vitreal protein levels during a two week period were unchanged from baseline levels. Thus, steroid treatment abolished the accumulation of protein in the

vitreous body which was observed in the untreated laser exposed group.

In both the untreated noncoherent and coherent light exposed eyes the relationship between vitreal PGE_2 content, vitreal protein levels, and PGE_2 production by retina/choroid as depicted in graph 1 was similar. During the first week after laser exposure, PGE_2 production and vitreal PGE_2 levels were not related. However,during the second week, the second peak in PGE_2 production observed in both groups was associated with a coinciding elevation in vitreal PGE_2 content.

Changes in protein vitreal levels in the untreated laser group did not coincide with changes in PGE_2 vitreal content; as PGE_2 in the vitreous peaked on day 7, while vitreal protein levels peaked twice and at different time intervals (day 3 and 14), (Graph 1). Likewise, in the noncoherent light exposed eyes, changes in vitreal protein levels were not closely associated with vitreal PGE_2 content.

Of the three parameters studied,in the steroid treated group, it was PGE_2 production which peaked during the second week, while the other two variables remained unchanged from baseline. Thus,in the steroid treated group elevation of PGE_2 production was not associated with an accumulation in the vitreous of either PGE_2 or protein.

Summary and Conclusion.

In our study we investigated the effect of steroid treatment of eyes with a single argon laser induced retinal burn on changes in PGE_2

production by the retina/choroid, its accumulation in the vitreous as well as vitreal protein levels indicative of blood retinal barrier (BRB) permeability changes. This study also compared the response of a single argon laser retinal burn, with that of the noncoherent light exposure, used during laser irradiation.

PGE_2 production by the retina/choroid: it is well established that noncoherent light exposure causes an increase of eicosanoid production due to light induced lipid peroxidation of the rod outer segment (4-7). In our study, nonchorent light exposure was associated with a biphasic enhancement in PGE_2 production by the retina/choroid during a two week period; an initial peak on day 1 and a higher peak on day 7, were separated by a brief period of normal levels. This observation is in disagreement with a recent study (4) showing in nocoherant light exposed rats eyes an increased synthesis of hydroxeicosatetraenoic acid (HETE) and leukotrienes B_4 by the retina, but without an associated change in prostaglandins production.

In our study, the increased PGE_2 production in the noncoherent light exposed group might be due to the fact that the light source used by us was possibly stronger than the 100 FTC flourescent light used by Birkle et al (4), as well as to species differences and variability in the time intervals studied. Also, pre-laser procedure of contact lens fitting and pupil dilatation might play a partial role in inducing the intial elevation of PG's production.

PG involvement following rabbit's iris irradiation by various laser modalities is characterized by an increased release of PGE_2 into the aqueous humor (8-11). Nevertheless, no data is yet available on eicosanoid production following laser induced retinal burns.

Retinal laser irradiation in our study caused a enhancement in PGE_2 production which followed a pattern similar to that elicited by the noncoherent light source but with peaks of higher magnitude. A laser induced retinal trauma might be expected to elicit a response similar to that of the nervous system whose reaction to trauma is characterized by an enhancement in eicosanoids production (12-14). Thus, the retinal PGE_2 response in the laser group might be due to the combined effect of both the noncoherent light exposure and the laser induced trauma. Also, polymorphonuclear cell infiltration of the inflammatory area surrounding the laser lesion might also serve as additional site of PGE_2 production. (15).

Vitreal PGE_2 levels: In our study, a transitory accumulation of PGE_2 in the vitreous body to levels above baseline occurred similarly in both the noncoherent light and laser exposed groups. This elevation in vitreal PGE_2 content might be partly due to the fact that PG produced by the retina are not stored but are immediately released into the vitreous body. In addition PGE_2 vitreal accumulation is determined by the rate of their removal out of the vitreous body by an active transport mechanism located at the ciliary processes (18-20)

and possibly at the BRB (16) both of which under normal conditions do not allow PG accumulation above baseline levels.

Indeed, a blockade of the anterior uveal PGE_2 absoptive mechanism was demonstrated following an episode of uveitis resulting in PGE_2 accumulation in the aqueous humor (19). Similarly, it has already been suggested (17) that the BRB located PGE_2 removal mechanism might be blocked following an inflammatory reaction with a subsequent increase in PGE_2 vitreal content.

In the present study, the role of transitory changes in the PGE_2 absorptive mechanism from the vitreous body following either the noncoherent light or the laser exposure are as yet unclear.

Vitreal protein levels: In the present study, vitreal protein levels in both the laser and the noncoherent light exposed eyes, were transiently elevated above baseline sometime during follow up period indicating a breakdown of the BRB. However, vitreal protein levels in the laser group peaked twice, as compared with a single peak in the other group.

The breakdown of the BRB following laser exposure has so far been demonstrated by the leakage of florescein, into the vitreous body (20) and by various histologic technique (21) and has been associate with elevated vitreal PGE_2 levels (26). Our study serves as direct evidence for protein leakage into the vitreous body BRB, yet the enhancement in vitreal PGE_2 levels above baseline in both our laser and the noncoherent light exposed groups did not coincide with

in protein leakage into the vitreous. This might be indicative of non PGE_2 mediated mechanism (22) responsible for BRB disruption in these groups and/or that vitreal PGE_2 levels were too low to affect the barrier. Indeed, a previous study (23) has shown that the BRB remained intact when the amounts of PGE_2 injected intravitreally did not exceed 200 μg/ml levels which were higher by 4 orders of magnitude from the highest vitreal PGE_2 content in our study.

Steroid treatment of laser exposed eyes inhibited PGE_2 and protein accumulation in the vitreous to levels above baseline during a two week period. This is related to steroid effect on PG production but might also indicate, as yet unknown, favorable effect of steroids on the BRB. Maintenance of normal vitreal PGE_2 and protein levels throughout the two week follow up period in steroid treated eyes, indicates the efficacy of this treatment in inhibiting the laser induced inflammatory reaction. Steroid treatment of the laser exposed eyes, also inhibited the initial increase in PGE_2 production, yet it did not prevent the development of a later peak observed also in the untreated laser exposed eyes. The abolishment of the initial peak is in accordance with the inhibitory effect of steroids on PG production due to their effect on reduction of the availability of arachidonic acid. However, augmentation of PGE_2 production in our steroid treated group in the late phase is as yet unclear.

Conclusive remarks

Vitreal PGE_2 and protein content: accumulation of PGE_2 and protein in the vitreous, a phenomemon not occurring in normal eyes is the manifestation most indicative of the inflammatory reaction following laser induced retinal trauma. Both PGE_2 and protein accumulation in the laser exposed eyes are the result of a "break" in the BRB characteristic of most inflammatory reactions. A progressive accumulation of PGE_2 in the vitreous has an adverse effect on the retina.

PGE_2 production by retina/choroid: In contrast to PGE_2 and protein vitreal content, parameters measured in vivo, PGE_2 production by retina/choroid is determined in vitro, limiting its applicability as a parameter of the inflammatory reaction.

However, our observation of an increase in PGE_2 production by the retina/choroid following argon laser induced retinal trauma, which was higher than that of noncoherent light exposed eyes might indicate that the augmented response in the laser group might be due to the combined effect of both the noncoherent light exposure and the laser induced trauma.

Steroid treatment of laser exposed eyes - Steroid treatment effectively inhibited the inflammatory reaction caused by the laser induced retinal damage as in the treated group normal vitreal PGE_2 and protein levels were maintained throughout a two week follow up period. The inhibitory effect of steroid treatment on the inflammatory reaction in laser exposed eyes was related to reduction in PGE_2

production by the retina/choroid, at the initial phase after exposure, thus indicating the efficacy of steroid treatment when started immidiately after exposure. However, steroid effect on the later stages of the inflammatory reaction following laser exposure are as yet unclear.

Clinical implication and suggestions for further investigation: Steroid treatment is effective in prevention of the inflammatory response following laser trauma, but other medications should be sought for complete inhibition of PGs production at the later stages after exposure.

A better inhibition of PG production may be achieved by using various other nonsteroidal antiinflammatory drugs, alone or in combination with steroids, which requires further studying.

2. Study on leukotrienes involvement in a single argon laser induced retinal burn

   Methodology

   Argon laser exposure - same as in steroid study.

   Sample preparation - same as in steroid study.

   Protein determination - same as in steroid study.

   Leukotriene B_4 (LTB_4) determination - LTB_4 was determined using a specific radioimmunioassay kit (Amersham Co.) following an incubation procedure similar to that in the steroid study.

   Experimental design - 60 rabbits with a single argon laser induced retinal burn were divided into 4 subgroups:

a) 15 rabbits studied 1 day after exposure.

b) 15 rabbits studied 3 days after exposure.

c) 15 rabbits studied 7 days after exposure.

d) 15 rabbits studied 14 days after exposure.

Results

LTB_4 production by retina/choroid - LTB_4 production by retina/choroid of the argon laser exposed eyes during a two week period were very low and undetectable by our radioimmunoassay technique. Thus, they were similar to baseline levels, which were considered as 0. (See baseline levels in Nd:YAG laser study).

Vitreal LTB_4 content - Vitreal LTB_4 content of argon laser exposed eyes during the 2 weeks period peaked only on day 7 with an average of 71 ± 20 pg/gm weight.

Conclusive remark - Argon laser exposure was associated with a transient increase in vitreal LTB_4 content, but without a detectable increase in LTB_4 production by retina/choroid. This is in contrast with a prolonged augmentation in vitreal LTB_4 content and production observed in Nd:YAG laser exposed eyes (discussed later).

3. Histopathological changes in an argon laser inducted retinal burn.

One hour after laser exposure: At the first hour after laser exposure, the sensory retina at the lesion site is adherent to the retinal pigment epithelium (RPE) while in the surrounding area edema causes displacement of the retina from the RPE (Fig.1). The retinal layers at the laser lesion site showed a varied reaction to the laser

induced trauma. The photoreceptor layer was more damaged than the nerve fiber layer, while the choroid underlying the lesion was not destroyed but infiltrated by numerous polymorphonuclear (PMN) cells.

Day 1 after exposure: The laser lesion site appearance on day 1 after exposure, was unchanged from that observed at the first hour, except for an PMN infiltration of the photoreceptor layer not present before. The number of PMN in the choroid was markedly reduced compared to the first hour (Fig.2).

Day 3 after exposure: On day 3 the retina at the lesion center revealed necrosis with only "cell shadows" left. Pigment laden macrophages, not observed thus far, infiltrated the periphery of the laser lesion, and the PMN, which thus far comprised most of the choroidal cellular infiltrate, were now replaced by chronic inflammatory cells. (fig. 3).

Day 7 after exposure: On day 7 the retina and choroid underlying the lesion site showed scarring with associated damage to the RPE. Pigment laden macrophages were dispersed throughout the retinal layers, while at the damaged choroid chronic inflammatory cells predominated (Fig. 4).

Conclusive remarks

The histology of a retinal argon laser lesion in our study is similar to that observed by MARSHALL and MALLERIO (1,2).

Our observation, during the first day after exposure of the choroidal PMN infiltrate migration into the underlying outer retinal

layers is of interest as it was not mentioned so far. The presence of machrophage in our three day old laser lesion correlated well with MARSHALL and MELLERIO study in Ruby irradiated eyes (3), showing macrophage dispersion on days 4-5. Likewise, the presence of pigment laden macrophages in our one month old lesion is in accord with the latter report (3) indicating a late inflammatory reaction in laser induced retinal trauma. Macrophages as well as PMN are known to produce various eicosanoids and might serve as an active source of PGE_2 even long after laser irradiation.

4. Study on a single neodymium;YAG laser induced retinal burn - prostanoid and leukorienes B_4 involvement.

Methodoloy:

Animals - Same as in the argon laser study.

Noncoherent light exposure (Sham exposure) - consisted of 68 non-coherent light exposed rabbits already described in the steroid treatment study.

Neodymium:YAG laser procedure - A Neodymium : YAG (Nd:YAG) (Lasag:Thun Switzerland) was used, and the laser beam was focused through a Russel-Fankhauser retinal contact lens. A single laser burst was applied on the retina nasally to the optic disc using a single pulse of a single burst at the multimode mode with a power setting of 1.0 millijoules, which resulted in bubble formation on the retina surface. Energy levels were chosen so as not to get vitreal bleeding and were

similar in both the PGE_2 and LTB_4 study (group 3 and 4 in the experimental design).

Sample preparation - Same as in the steroid study.

Prostaglandin E_2 and protein determination - Same as in the steroid study.

Leukotrienes B_4 determination - LTB_4 was determined using a specific radioimmunoassay kit (Amersham Co.) following sample preparation and incubation procedure similar to that in the steroid study.

Experimental design - The study consisted of four groups of rabbits.

1) Control group - 30 rabbits used during the steroid study, which were not exposed to either the laser irradiation or the noncoherent light irradiation and had no ocular pathology.

2) Noncoherent light exposed group (Sham group) - 72 rabbits used during the steroid study which underwent sham exposure with the noncoherent light of the slit lamp and were divided into 4 subgroups:

   a) 21 rabbits studied 1 day after exposure.

   b) 10 rabbits studied 3 days after exposure.

   c) 10 rabbits studied 7 days after exposure.

   d) 31 rabbits studied 14 days after exposure.

3) Nd:YAG exposed group (PGE_2 response) - 31 rabbits with a single Nd:YAG laser induced retinal burn, in which PGE_2 response was studied, and were divided into four subgroups accordingly.

a) 12 rabbits studied 1 day after exposure.

b) 6 rabbits studied 3 days after exposure.

c) 6 rabbits studied 7 days after exposure.

d) 7 rabbits studied 14 days after exposure.

4) Nd:YAG exposed group (LTB_4 response) - 26 rabbits with a single Nd:YAG induced retinal burn in which LTB_4 response was studied, and were divided into 4 groups.

a) 11 rabbits studied 1 day after exposure.

b) 6 rabbits studied 3 days after exposure.

c) 5 rabbits studied 7 days after exposure.

d) 4 rabbits studied 14 days after exposure.

Results

Vitreal PGE_2 content Table 4): Vitreal PGE_2 content in the Nd:YAG laser irradiated eyes at each of the time intervals studied during a two week follow- up period were higher than baseline, with the highest levels noted on day 1 and 7 (15478 $\pm$ 5366 pg/gm weight and 13649 $\pm$ 5514 pg/gm weight, respectively).

Changes in vitreal PGE_2 content of the noncoherent light exposed eyes followed a course different from that observed in the laser exposed eyes, as they were elevated only once, on day 7 (13460 $\pm$ 5129 pg/gm weight).

PGE_2 production by retina/choroid (Table 5): PGE_2 production levels were augmented above baseline levels during a two week period following Nd:YAG laser exposure. However, levels at the first week

(1561 ± 505 pg/mg weight at day 1, 1593 ± 441 pg/mg weight, at day 3) were higher than those observed during the second week (724 ± 193 pg/mg weight at day 7 and 576 ± 10 pg/mg weight at day 14, respectively).

PGE_2 production by the retina/choroid of the noncoherent light exposed eyes showed, during a two week period, a biphasic elevation above baseline levels (already discussed in the steroid study). PGE_2 production peak values of the noncoherent light exposed eyes were each significantly lower than the corresponding Nd:YAG laser group.

Vitreal Protein Content (Table 6) - Vitreal protein content during a two week period following Nd:YAG laser exposure was elevated on day 7 and 14, and reached values of 0.72 ± 0.19 ug/mg weight and 0.8 ± 0.22 µg/mg weight respectively, which were significantly higher than baseline levels of 0.57 ± 0.3 µg/mg weight ($p< 0.06$ and $p<0.0013$ respectively). In the noncoherent light exposed eyes, vitreal protein levels were elevated only on day 1 after exposure (1.03 ± 0.2 µg/mg, $p<0.000$). and then returned to baseline levels.

In the noncoherent light exposed group the relationship betweeen vitreal PGE_2 content, vitreal protein levels and PGE_2 production by retina/choroid have been already discussed (in the steroid study).

In the Nd:YAG laser exposed group PGE_2 production was not closely related to vitreal PGE_2 levels, also vitreal protein levels in this

group which peaked on day 7 and 14 did not coincide with vitreal PGE_2 levels who were at their lowest.

LTB_4 levels in control eyes; LTB_4 production by the retina/choroid of the control groups was considered as baseline and was so low that it could not be detected with the radioimmunoassay in our use which is sensitive to levels higher the 6 pg/ml. The use of tissues from two eyes in each vial failed to achieve detectable levels, therefore, for practical purposes we assume that baseline levels LTB_4 production by retina/choroid approximated zero. Likewise baseline vitreal LTB_4 levels approximated zero.

LTB_4 production by the retina/choroid (Table 7)

LTB_4 production by retina-choroid of eyes subjected to a single Nd:YAG laser burn during a two week follow up period were unchanged from baseline levels.

LTB_4 vitreal levels in the laser exposed eyes were elevated compared to baseline levels throughout a two week period, but were highest during the second week (209 $\pm$ 77 pg/mg weight on day 7, and 59 $\pm$ 19 pg/mg weight on day 14) as the late augmentation in vitreal LTB_4 on day 7 reached levels 3 fold higher than the increase.

Protein vitreal content -vitreal protein levels in Nd:YAG treated eyes exceeded control levels 0.57 $\pm$ 0.44 ng/mg weight during the second week after exposure (0.72 $\pm$ 9 ng/mg weight and 0.84 $\pm$ 0.22 ng/mg weight respectively), $p<0.02$, and $p<0.013$.

Summary and Conclusion:

Nd:YAG exposed eyes - PGE_2 response.

Nd:YAG retinal burn was associated with an inflammatory reaction of higher magnitude and longer duration than that of the argon exposed eyes as manifested by a persistant elevation of vitreal PGE_2 and protein levels during a period of 2 weeks.

Vitreal protein content, indicative of BRB disruption in the Nd:YAG exposed eye remained above baseline levels during the second week after exposure, as compared with a biphasic transient elevation in the argon group. Likewise the elevation in PGE_2 production by retina/choroid of Nd:YAG exposed eyes was maintained throughout the two week follow up period with higher levels during the first week after exposure as compared with a late transient elevation in the argon group.

LTB_4 response to argon and Nd:YAG laser exposure:

A single induced Nd:YAG laser retinal burn was associated with a significant accumulation of LTB_4 in the vitreous which persisted during a two week period which was more pronounced than that of the argon group where levels peaked only once.

Thus, Nd:YAG induced retinal damage was associated with a more pronounced inflammatory reaction then those in argon exposed eyes as manifested by higer vitreal PGE_2, LTB_4 and protein levels. In view of this observation a more effective antiinflammatory therapy of Nd:YAG

laser exposed eyes directed at inhibiting LTB_4 production should be considered.

Clinical significance and suggestion for further investigation

The clinical significance of our study has four aspects:

1)Steroid treatment used by us, effectively inhibited the inflammatory reaction when started immediately after laser exposure. This effect was related to an initial supression of PGE_2 production. Other medications should be sought for complete inhibition of the later phases of the inflammatory reaction.

2)Laser induced leukotirene response was of higher magnitude and longer duration than prostaglandin response, indicating the importance of their mediatory role in the laser induced inflammatory reaction and the need for a search for antileukotriene drug as a possible therapy for the laser induced retinal injury.

3)The inflammatory response associated with Nd:YAG laser exposure as shown for the first time in this study was more pronounced than that induced by argon laser exposure. This has military significance as most expected military eye injury are likely to be caused by the Nd:YAG laser.

4)The Nd:YAG laser induced inflammatory reaction in our study was evident throughout a two week period after exposure indicating that follow up of laser exposed patients, especially those with previous ocular inflammation trauma or diabetes, should extend over two weeks.

This observation is relevant to current ophthalmic laser practices as in glaucoma treatment (laser iridotomy) or cataract surgery (capsulotomy).

The clinical significance of our study lies in the demonstration of the efficacy of steroid treatment laser induced inflammatory reaction. However, our finding on Nd:YAG laser exposure might be of military importance, as the Nd:YAG laser induced retinal damage is liable to be the most expected military induced eye injury. The finding that the Nd:YAG laser induce a severe inflammatory reaction with a pronounced leukotrienes response might have a bearing on future studies indicating that antileukotriene drugs should be sought for their treatment.

Table 1

Effect of steroid treatment on prostaglandin E_2 production by the retina/choroid following a single argon laser induced retinal lesion - comparison with the effect of noncoherent light exposure.

	Prostaglandin E_2 production by retina/choroid (pg/mg weight) mean ±SD				
Time after exposure (days)	noncoherent light exposured group	untreated laser group	steroid treated laser group	* p(L) Value	** p Value(s)
1	507±261 *** n=22	778±232 n=19	262±127 n=12	0.0006	0.0000
3	363±129 n=14	452±235 n=22	397±164 n=20	****N.S.	N.S.
7	982±317 n=12	1971±291 n=10	1260±514 n=11	0.0001	0.000
14	534±245 n=28	637±267 n=19	1934±745 n=11	N.S.	0.000
baseline	360±137 n=30				

*P (L) p value for t-student test comparing each of the noncoherent light exposed eyes at each time interval with the corresponding untreated laser exposed groups.

**P (S) p-value for t-student test comparing the untreated laser exposed eyes at each time interval with the corresponding steroid treated group.

*** n n indicates number of eyes involved in each group.

****N.S. statistically not significant

Table 2

Effect of steroid treatment on vitreal prostaglandin E_2 levels following a single argon laser induced retinal lesion - a comparison with noncoherent light exposed eyes.

	Prostaglandin E_2 vitreal levels (pg/gm weight) mean ±SD				
Time after exposure (days)	noncoherent light exposed group	untreated laser group	steroid treated laser group	* p Value(L)	** p Value(s)
					****
1	7381±3238 n=21	4654±2141 n=20	4401±1594 n=12	0.0003	N.S.
3	7243±2968 n=10	3791±2137 n=20	5753±2723 n=22	0.0004	0.0014
7	13460±5129 n=10	13668±7125 n=10	7668±2844 n=11	N.S.	0.002
14	4666±2374 n=31	4660±2447 n=23	5083±1900 n=12	N.S.	N.S.
baseline	7476±4770 n=30				

*P (L) p value for t-student test camparing the noncoherent light exposed eyes at each time interval with the corresponding untreated laser exposed group

**P (s) p value for t-student test comparing each of the laser untreated eyes at each time interval with the corresponding steroid treated group.

***n n indicates number of eyes involved in the each group.

***N.S. statistically not significant.

Table 3

Effect of steroid treatment on vitreal protein levels following a single argon laser induced retinal burn - a comparison with noncoherent light exposed eyes.

Time after exposure (days)	Vitreal protein levels (µg/mg weight) mean ±SD: noncoherent light exposed group	untreated laser group	steroid treated laser group	* P Value(L)	** P Value(s)
1	1.03±0.2 *** n=27	0.63±0.17 n=20	0.50±0.15 n=18	0.01	N.S.
3	0.69±0.19 n=18	0.83±0.24 n=22	0.58±.17 n=22	****N.S.	N.S.
7	0.63±0.14 n=12	0.55±2 n=10	1.23±0.31 n=10	N.S.	0.0000
14	0.73±0.2 n=31	0.99±0.21 n=23	0.54±0.17 n=12	N.S.	0.0001
baseline	0.57±0.24				

*P (L) p value for t-student test camparing noncoherent light exposed eyes at each of the time intervals with the corresponding untreated laser exposed groups.

**P (s) p value for t-student test comparing each of the laser untreated eyes at each of the time intervals with the corresponding steroid treated group.

***n n indicates number of eyes involved in the involved group.

***N.S. statistically not significant.

Table 4

Vitreal prostaglandin E_2 changes following a single Neodymiun:YAG laser retinal exposure - Comparison with noncoherent light exposure.

	Vitreal prostaglindin E_2 (pg/gr weight) (mean±SD)[2]		
Time after exposure (days)	ND:YAG laser group	noncoherent light exposed group	* P value
1	15478±5366 ** n=11	7381±5238 n=21	0.0000
3	10664±3155 n=6	7245±2968 n=6	0.02
7	13649±5514 n=6	13460±5129 n=10	N.S.
14	11535±713 n=7	4666±2374 n=31	0.0000
Baseline	7476±4770 n=30		

* p -p value for t-student test comparing Neodymiun-YAG group with the corresponding noncoherent group.

** n -indicates number of eyes involved in each group.

Table 5

Prostaglandin E_2 production by the retina/choroid following Neodymiun:YAG laser induced retinal lesion-comparison with noncoherent light exposure

Time after exposure (days)	Prostaglindin E_2 production by retina choroid (pg/mg weight) (mean±SD) ND:YAG laser group	noncoherent light exposed group	*P value
1	1561±505 ** n=12	507±261 n=27	0.0000
3	1593±441 n=8	363±129 n=14	0.0000
7	724±193 n=5	982±327 n=8	***N.S.
14	576±10 n=4	534±245 n=31	N.S.
Baseline	360±137 n=30		

* p -p value for t-student test comparing Neodymiun-YAG eye at each time interval with the corresponding noncoherent light exposed group.

** n -indicates number of eyes involved in each group.

***N.S. statistically not significant.

Table 6

Vitreal protein levels following a single Neodymiun:YAG laser retinal burn.

Vitreal protein levels (μg/mg weight) mean $\pm$ SD.			
Time after exposure (days)	noncoherent light exposed group	ND:YAG laser group	** P-Value
1	1.03$\pm$0.2 *n=27	0.63$\pm$0.20 n=13	***N.S.
3	0.69$\pm$0.19 n=18	0.57$\pm$016 n=7	N.S.
7	0.63$\pm$0.14 n=12	0.72$\pm$0.19 n=5	N.S.
14	0.73$\pm$0.2 n=31	0.84$\pm$0.22 n=5	0.0013
Baseline	0.57$\pm$0.14 n=9		

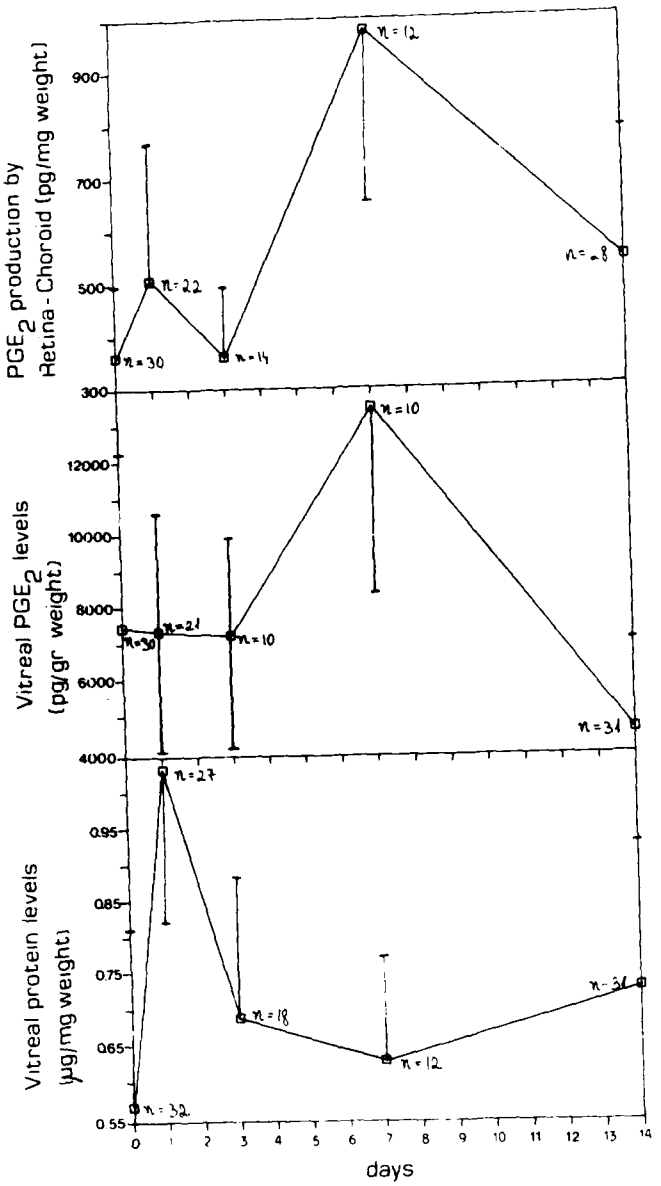
* n -indicates number of eyes involved in each group.
** p -p value for t-student test comparing Neodymiun-YAG group with the corresponding light group.
*** N.S.-Statistically not significant.

Table 7

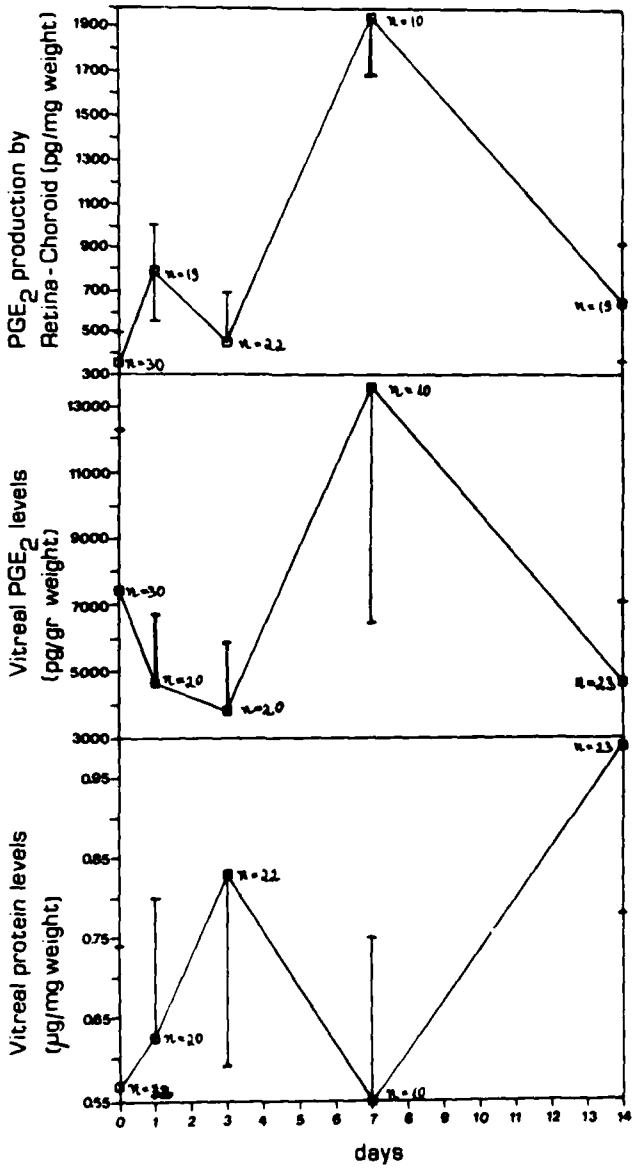
The effect of a single Neodymiun:YAG Laser induced retinal burn on leukotriene B_4 (LTB_4) production by the retina/choroid and its accumulation in the vitreous body.

Time after exposure (days)	LTB_4 production by retina/choroid (pg/mg weight) mean+SD	Vitreal LTB_4 level pg/gm weight mean+SD
1	*(-) n=12	53±21 n=11
3	(-) n=8	59±19 n=6
7	(-) n=5	209±77 n=5
14	(-) n=4	124±17 n=4
baseline	(-)	(-)

-* the dash sign indicates low levels undetected by radioimmunoassay.
**n= n indicates number of eyes involved in each group.



Graph 1 The association between PGE_2 by retina/choroid vitreal PGE_2 levels and vitreal protein level, over a two week period following noncoherent light exposure (Sham Exposure). The values shown are the mean of the number of eyes involved in each group as indicated by U. No error but were added as they have already mentioned in the appropriate tables. RTC indicate PGE_2 production by the retina/choroid.



Graph 2 The association between PGE_2 production by retina/choroid, vitreal PGE_2 levels and vitreal protein levels over a two week period following a single argon laser retinal burn. The values shown are the mean of the number of eyes involved in each group as indicated by the N. No error but were added as they have already mentioned in the appropriate tables. RtC indicate PGE_2 production by the retina/choroid.

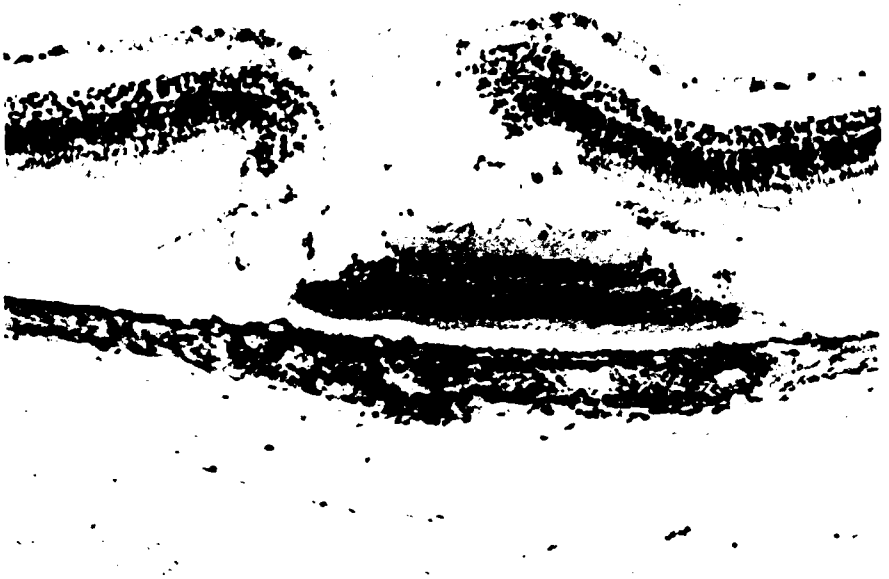


Fig. 1. One hour after laser treatment. The sensory retina at the laser lesion center, is "adherent" to the retinal pigment epithel. (Hematoxylin and Eosin x 100).

Fig. 2. Twenty four hours after laser exposure. Massive infiltration of polymorphonuclear cells is seen in the photoreceptor layer.

Fig. 3. Three days after laser exposure. The adherent retina undergoes necrosis, and the periphery of the laser lesion sites is infiltrated by macrophages.

Fig. 4. Seven days after exposure. The retina is scarred and infiltrated by pigment laden macrophages. The retinal pigment epithal is completely destroyed.

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